FILE 'CAPLUS, MEDLINE, BIOSIS, CA, SCISEARCH, EMBASE' ENTERED AT 09:01:48 ON 09 APR 2003

L1	18	S	FINK/AU
L2	12		DUPLICATE REM L1 (6 DUPLICATES REMOVED)
L3	12951	S	MICRODISSECTION
L4	254453	S	IMMUNOFLUORESCENCE
L5	107	S	L3 AND L4
L6	35		DUPLICATE REM L5 (72 DUPLICATES REMOVED)
L7	13140130	S	TISSUE OR CELL
L8	25	S	L6 AND L7
L9	1290335	S	LASER
L10	8	S	L8 AND L9

ANSWER 2 OF 8 CAPLUS COPYRIGHT 2003 ACS

TI Identification of nitric oxide synthase neurons for **laser** capture **microdissection** and mRNA quantification

AB An immunohistochem. technique was developed to visualize nitric oxide synthase (NOS)-immunopos. neurons in fresh-frozen **tissue** sections of rat brain for **laser** capture **microdissection** (LCM) and mRNA anal. The effect of **tissue** fixation and the choice of fluorophore were investigated. Here we describe a rapid **immunofluorescence** protocol that allows the processing of fresh-frozen **tissue** sections within eight minutes and subsequent mRNA extn. and real-time PCR from pools of 20 NOS-immunopos. LCM neurons. The cellular complement of a subset of ionotropic glutamate receptors, specifically N-methyl-D-aspartate receptor subunit mRNAs, was examd. because these receptor complexes are thought to mediate the effects of fast and slow glutamate excitotoxicity. Real-time PCR data revealed that striatal NOS interneurons express the mRNAs encoding NR1, NR2A, NR2B, and NR2D but not NR2C. These LCM mRNA data are consistent with previous in situ hybridization studies and demonstrate the utility of rapid immuno-LCM with real-time quant. PCR for the study of mRNA abundance in discrete populations of neurons within the mammalian brain.

SO BioTechniques (2002), 33(6), 1274-1278, 1280-1283
CODEN: BTNQDO; ISSN: 0736-6205

AU Bi, Wenya Linda; Keller-McGandy, Christine; Standaert, David G.; Augood, Sarah J.

L10 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2003 ACS

TI Immunostaining for **cell** picking and real-time mRNA quantitation

AB **Microdissection** techniques allow a **cell**-type or even **cell**-specific mRNA anal. within complex tissues. Furthermore, valid mRNA quantitation can be performed by real-time reverse transcriptase-polymerase chain reaction from a few isolated cells obtained from cryosections. For a more precise access to many **cell** types, this technique has to be complemented by a **cell** -type-specific immunostaining. To evaluate its effect on mRNA quantitation, we analyzed alveolar macrophages (AMs) from control rat lungs and those undergoing stimulation with lipopolysaccharide and interferon-.gamma. nebulization. Whereas AMs from the left lung were directly harvested for mRNA extn. by bronchoalveolar lavage, **tissue** sections of the right lung were stained with an optimized **immunofluorescence** protocol detecting AMs. Fifteen AM profiles per sample were picked by **laser**-assisted sampling technique. Normalizing to a std. gene, nitric oxide synthase II (NOSII) and tumor necrosis factor (TNF)-.alpha. mRNA were quantified by real-time reverse transcriptase-polymerase chain reaction. In stimulated lungs, the percentage of picked samples pos. for NOSII or TNF-.alpha. mRNA increased significantly. Moreover, a marked increase in the ratio of target gene mRNA to std. gene mRNA was noted for both NOSII and TNF-.alpha. in picked AMs from stimulated lungs, which matched very well the increase detected in the lavaged AMs undergoing direct RNA extn. Thus, when using an optimized protocol for **immunofluorescence**, this approach may be reliably combined with **laser**-assisted **cell** picking and real-time mRNA quantitation in a few immunohistochem. characterized **cell** profiles within complex tissues.

SO American Journal of Pathology (2000), 157(5), 1459-1466
CODEN: AJPAA4; ISSN: 0002-9440

AU Fink, Ludger; Kinfe, Thomas; Seeger, Werner; Ermert, Leander; Kummer, Wolfgang; Bohle, Rainer Maria

L10 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2003 ACS

TI IF-LCM: **laser** capture **microdissection** of immunofluorescently defined cells for mRNA analysis rapid communication

AB The next phase of the mol. revolution will bring functional genomics down to the level of individual cells in a **tissue**. **Laser** capture **microdissection** (LCM) coupled with reverse transcription-polymerase chain reaction (RT-PCR) can measure gene expression in normal, cancerous, injured, or fibrotic **tissue**. Nevertheless, targeting of specific cells may be difficult using routine morphol. stains. Immunohistochem. can identify cells with specific antigens; however, exposure to aq. solns. destroys 99% of the mRNA. Consequently, there is an overwhelming need to identify specific **tissue** cells for LCM without mRNA loss. We report on a rapid immunofluorescent LCM (IF-LCM) procedure that allows targeted anal. of gene expression. A LCM microscope was outfitted for epifluorescence and light level video microscopy. Heat filters were added to shield the image intensifier from the **laser**. Frozen sections were fluorescently labeled by a rapid one minute incubation with anti-Tamm-Horsfall antibody and an ALEXA-linked secondary antibody. Fluorescently labeled thick ascending limb (TAL) cells were detected by low light level video microscopy, captured by LCM, and mRNA was analyzed by RT-PCR for basic amino acid transporter, Tamm-Horsfall protein, and aquaporin-2. The immunofluorescently identified TAL could be cleanly microdissected without contamination from surrounding tubules. The recovery of RNA following rapid **immunofluorescence** staining was similar to that obtained following hematoxylin and eosin staining, as assessed by RT-PCR for malate dehydrogenase. We conclude that the new app. and method for the immunofluorescent labeling of **tissue** cells targeted for LCM can isolate pure populations of targeted cells from a sea of surrounding cells with highly acceptable preservation of mRNA. Since the TAL is minimally injured following ischemia, identification of the different responses between TAL and surrounding **tissue** in damaged kidneys may provide new therapeutic targets or agents for the treatment of acute renal failure.

SO Kidney International (2000), 58(3), 1346-1353
CODEN: KDYIA5; ISSN: 0085-2538

AU Murakami, Hiroshi; Liotta, Lance; Star, Robert A.

L10 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2003 ACS

TI Immunostaining and **laser**-assisted **cell** picking for mRNA analysis

AB Isolation of single cells or **cell** clusters from complex **tissue** sections has become possible by **microdissection** techniques. Employing **laser**-assisted **cell** picking, **cell**-specific mRNA anal. of a few isolated **cell** profiles may be performed. However, microscopic discrimination of different **cell** types in routinely stained **tissue** sections is limited, whereas immunostaining enables a more precise access to cells of interest. This approach was noted to interfere with mRNA recovery. To define optimal conditions for mRNA amplification from immunodetected cells, we systematically investigated several potential affectors. Kind of fixation, antibodies and staining reagents, incubation and total processing time, and digestion with proteinase K turned out to influence mRNA stability. The authors present rapid protocols for immunohistochem. and **immunofluorescence** with total incubation times of approx. 25 to 40 min and 10 to 20 min, resp., and suggest mRNA amplification without a preceding extn. step. Applying these protocols to oligocellular clusters contg. approx. 20 **cell** profiles and nuclei each from lung and kidney **tissue**, the highest efficiency rates of mRNA amplification were obtained when combining short-term formalin fixation, redn. of antibody incubation time, application of **immunofluorescence**, and digestion with proteinase K. Thus, the successful combination of immunostaining and **laser**-assisted **cell** picking remarkably improves **cell** type-specific anal. of gene expression within complex tissues.

SO Laboratory Investigation (2000), 80(3), 327-333
CODEN: LAINAW; ISSN: 0023-6837

AU Fink, Ludger; Kinfe, Thomas; Stein, Maria Magdalena; Ermert, Leander; Hanze, Jorg; Kummer, Wolfgang; Seeger, Werner; Bohle, Rainer Maria

L4 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
AN 2000:673285 CAPLUS
DN 134:159615
TI IF-LCM: laser capture microdissection of immunofluorescently defined cells for mRNA analysis rapid communication
AU Murakami, Hiroshi; Liotta, Lance; Star, Robert A.
CS Renal Diagnostics and Therapeutics Unit, National Institutes of Health, Bethesda, MD, USA
SO Kidney International (2000), 58(3), 1346-1353
CODEN: KDYIA5; ISSN: 0085-2538
PB Blackwell Science, Inc.
DT Journal
LA English
RE.CNT 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

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(FILE 'HOME' ENTERED AT 09:37:28 ON 10 APR 2003)

FILE 'CAPLUS, MEDLINE, BIOSIS, CA, SCISEARCH, EMBASE' ENTERED AT 09:37:39 ON 10 APR 2003
L1 2210 S LASER (W) CAPTURE (W) MICRODIS?
L2 11463 S IMAGE (W) INTENSI?
L3 5 S L1 (S) L2
L4 1 DUPLICATE REM L3 (4 DUPLICATES REMOVED)